

## 1 Nomenclature

### EC number

3.1.1.81

### Systematic name

N-acyl-L-homoserine-lactone lactonohydrolase

### Recommended name

quorum-quenching N-acyl-homoserine lactonase

### Synonyms

AHL lactonase <6, 12, 13, 15, 16, 17, 18, 19, 20, 43> [1, 7, 12, 14]

AHL-degrading enzyme <6, 14, 38, 39, 42> [4, 8, 11]

AHL-inactivating enzyme <6, 12, 16, 17, 18, 19, 20, 43> [1]

AHL-lactonase <1, 2, 3, 4, 7, 9, 10, 13, 16, 17, 18, 19, 20, 35, 40, 42, 43> [1, 8, 9, 10, 12, 13, 15]

AHLase <38, 39> [11]

AhID <38> [11]

AhIK <39> [11]

AiiA <1, 2, 3, 4, 9, 10, 35, 40> [9, 15, 16]

AiiA lactonase <6, 7, 14> [4, 6]

AiiA-like protein <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> [2]

AiiB <11> [3]

AiiC <11> [3]

AttM <11> [3]

N-acyl homoserine lactonase <11, 35> [3, 13]

N-acyl homoserine lactone hydrolase <11> [3]

N-acyl-homoserine lactonase <13> [12]

N-acyl-homoserine lactone lactonase <13> [12]

N-acylhomoserine lactonase <38, 39> [11]

PvdQ <5> [5]

QuiP <5> [5]

acyl homoserine degrading enzyme <38> [11]

acyl-homoserine lactone acylase <5> [5]

delactonase <35> [13]

lactonase-like enzyme <1, 2, 3, 4, 9, 10> [9]

quorum-quenching N-acyl homoserine lactonase <6, 12, 16, 17, 18, 19, 20, 43> [1]

quorum-quenching N-acyl homoserine lactone hydrolase <15> [14]

quorum-quenching N-acyl homoserine lactone lactonase <7, 40> [10, 15]

quorum-quenching enzyme <7, 13, 35> [10, 12, 13]  
quorum-quenching lactonase <15> [7]  
quorum-sensing enzyme  
Additional information <13> (<13> the enzyme belongs to the quorum-quenching enzymes [12]) [12]

**CAS registry number**

389867-43-0

## 2 Source Organism

<1> *Mus musculus* (no sequence specified) [9]  
<2> *Homo sapiens* (no sequence specified) [9]  
<3> *Bos taurus* (no sequence specified) [9]  
<4> *Oryctolagus cuniculus* (no sequence specified) [9]  
<5> *Pseudomonas aeruginosa* (no sequence specified) [5]  
<6> *Bacillus cereus* (no sequence specified) [1,4]  
<7> *Bacillus* sp. (no sequence specified) [6,10]  
<8> no activity in *Bacillus sphaericus* [1]  
<9> *Capra hircus* (no sequence specified) [9]  
<10> *Equus caballus* (no sequence specified) [9]  
<11> *Agrobacterium tumefaciens* (no sequence specified) [3]  
<12> *Bacillus mycoides* (no sequence specified) [1]  
<13> *Ralstonia* sp. (no sequence specified) [12]  
<14> *Bacillus anthracis* (no sequence specified) [4]  
<15> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8B9) [2, 7, 14]  
<16> *Bacillus* sp. (UNIPROT accession number: Q8RPW9) [1]  
<17> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW7) [1]  
<18> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW6) [1]  
<19> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW5) [1]  
<20> *Bacillus thuringiensis* (UNIPROT accession number: Q8RJA0) [1]  
<21> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C1) [2]  
<22> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C2) [2]  
<23> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C0) [2]  
<24> *Bacillus thuringiensis* (UNIPROT accession number: Q7B8C4) [2]  
<25> *Bacillus thuringiensis* (UNIPROT accession number: Q7KI79) [2]  
<26> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTX1) [2]  
<27> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW4) [2]  
<28> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW7) [2]  
<29> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW6) [2]  
<30> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW8) [2]  
<31> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTX0) [2]  
<32> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTC3) [2]  
<33> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTC5) [2]  
<34> *Bacillus thuringiensis* (UNIPROT accession number: Q8KTW9) [2]  
<35> *Bacillus* sp. (UNIPROT accession number: Q9L8R8) [13, 16]

- <36> *Bacillus thuringiensis* (UNIPROT accession number: Q8K7W5) [2]
- <37> *Bacillus* sp. (UNIPROT accession number: Q8KTW3) [2]
- <38> *Arthrobacter* sp. (UNIPROT accession number: Q7X3T2) [11]
- <39> *Klebsiella pneumoniae* (UNIPROT accession number: Q7X477) [11]
- <40> *Bacillus thuringiensis* subsp. *kurstaki* (no sequence specified) [15]
- <41> no activity in *Gallus gallus serum* [9]
- <42> *Bacillus thuringiensis* subsp. *kurstaki* (UNIPROT accession number: Q7B8B9) [8]
- <43> *Bacillus thuringiensis* (UNIPROT accession number: Q8RPW8) [1]
- <44> no activity in *Bacillus fusiformis* (no sequence specified) [1]

### 3 Reaction and Specificity

#### Catalyzed reaction

an N-acyl-L-homoserine lactone + H<sub>2</sub>O = an N-acyl-L-homoserine (<15> active site structure and substrate binding, catalytic mechanism, overview [14]; <38,39> hydrolysis of the lactone ring [11]; <7> residues H106, D108, and H109, as well as H169 are important for catalytic activity [10]; <40> substrate binding and catalytic mechanism, overview [15])

#### Natural substrates and products

- S** N-3-oxododecanoyl-L-homoserine lactone + H<sub>2</sub>O <1, 2, 3, 4, 5, 9, 10> (<5> QuiP is required for utilization and growth on long-chain acylhomoserine lactones of *Pseudomonas aeruginosa*, while PvdQ is not [5]) (Reversibility: ?) [5, 9]
- P** N-3-oxododecanoyl-L-homoserine
- S** N-3-oxooctanoyl-L-homoserine lactone + H<sub>2</sub>O <11> (Reversibility: ?) [3]
- P** N-3-oxooctanoyl-L-homoserine
- S** N-acyl-(S)-homoserine lactone + H<sub>2</sub>O <6, 7, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 43> (<7> N-acylhomoserine lactones, AHL, regulate protease production, swarming motility, biofilm formation, and *Caenorhabditis elegans* killing efficiency in most strains of the Burkholderia complex organisms, which is inhibited by *Bacillus* AHL lactonase acting as a quorum-quenching enzyme [6]; <6,12,16,17,18,19,20,43> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence bacteria on plants [1]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora* strain IBN98 on *Solanum tuberosum*, acylhomoserine lactones are autoinducers of quorum-sensing signaling [2]; <38> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora* strain N98 [11]; <7, 35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*, acylhomoserine lactones are autoinducers of quorum-sensing signaling, mechanism [10, 16];

<35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*, acylhomoserine lactones are autoinducers of quorum-sensing signaling, the inhibition of which is a feasible approach for prevention of bacterial infection [13]; <39> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of pathogenic bacteria [11]; <11> the isoforms AttM, AiiB, and AiiC inactivate the acylhomoserine lactone quorum-sensing signaling and attenuate the virulence of *Erwinia carotovora* subsp. atroseptica strain 6276 on *Solanum tuberosum*, function and regulation of quorum-sensing signaling, overview [3]) (Reversibility: ?) [1, 2, 3, 6, 10, 11, 13, 16]

P N-acyl-(S)-homoserine

S N-decanoyl-L-homoserine lactone + H<sub>2</sub>O <5> (Reversibility: ?) [5]

P N-decanoyl-L-homoserine

S N-hexanoyl-L-homoserine lactone + H<sub>2</sub>O <11> (Reversibility: ?) [3]

P N-hexanoyl-L-homoserine

S Additional information <1, 2, 3, 4, 5, 7, 9, 10, 13, 15, 38, 39, 42> (<4> acylhomoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorum-quenching enzyme [9]; <1, 2, 3, 9, 10> acylhomoserine lactone quorum-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorum-quenching enzyme [9]; <7,15> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence [7, 10]; <5> N-acyl homoserine lactone quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence, regulation quorum-sensing signaling and quorum-quenching in *Pseudomonas aeruginosa* [5]; <42> N-acyl homoserine lactone, AHL, quorum-sensing signals are the vital elements of bacterial quorum-sensing systems, which regulate diverse biological functions, including virulence and biofilm formation of Gram-negative bacteria, the enzyme deactivates the signaling by degradation of AHLs via lactone ring hydrolysis [8]; <38, 39> quorum-sensing is a signaling mechanism, that controls diverse biological functions, including virulence, via N-acylhomoserine lactone signal molecules in Gram-negative bacteria, overview [11]; <13> the enzyme is involved in inactivation of N-acylhomoserine lactone signaling in regulation of infection and virulence functions [12]) (Reversibility: ?) [5, 7, 8, 9, 10, 11, 12]

P ?

#### Substrates and products

S N-3-hydroxybutanoyl-L-homoserine lactone + H<sub>2</sub>O <7> (Reversibility: ?) [10]

P N-3-hydroxybutanoyl-L-homoserine

S N-3-oxobutanoyl-L-homoserine lactone + H<sub>2</sub>O <7> (Reversibility: ?) [10]

P N-3-oxobutanoyl-L-homoserine

S N-3-oxodecanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 7, 35> (<4,7> high activity [9,10]) (Reversibility: ?) [9, 10, 16]

P N-3-oxodecanoyl-L-homoserine

S N-3-oxododecanoyl-L-homoserine lactone + H<sub>2</sub>O <1, 2, 3, 4, 5, 7, 9, 10, 35, 38> (<4> best substrate [9]; <5> QuiP is required for utilization and growth on long-chain acylhomoserine lactones of *Pseudomonas aeruginosa*, while PvdQis not [5]; <35> the substrate regulates production of virulence determinants of the human pathogen *Pseudomonas aeruginosa* [13]) (Reversibility: ?) [5, 9, 10, 11, 13]

P N-3-oxododecanoyl-L-homoserine (<35> product identification by mass spectrometry [13])

S N-3-oxohexanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 7, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39> (Reversibility: ?) [2, 9, 10, 11, 13, 16]

P N-3-oxohexanoyl-L-homoserine (<35> product identification by mass spectrometry [13])

S N-3-oxooctanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 7, 11, 35> (<7> high activity [10]; <35> the substrate controls Ti plasmid conjugal transfer in *Agrobacterium tumefaciens* [13]) (Reversibility: ?) [3, 9, 10, 13, 16]

P N-3-oxooctanoyl-L-homoserine (<35> product identification by mass spectrometry [13])

S N-acyl-(S)-homoserine lactone + H<sub>2</sub>O <6, 7, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 43> (<7> N-acylhomoserine lactones, AHL, regulate protease production, swarming motility, biofilm formation, and *Caenorhabditis elegans* killing efficiency in most strains of the *Burkholderia* complex organisms, which is inhibited by *Bacillus* AHL lactonase acting as a quorum-quenching enzyme [6]; <6, 12, 16, 17, 18, 19, 20, 43> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence bacteria on plants [1]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora* strain IBN98 on *Solanum tuberosum*, acylhomoserine lactones are autoinducers of quorum-sensing signaling [2]; <38> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora* strain N98 [11]; <7, 35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*, acylhomoserine lactones are autoinducers of quorum-sensing signaling, mechanism [10, 16]; <35> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*, acylhomoserine lactones are autoinducers of quorum-sensing signaling, the inhibition of which is a feasible approach for prevention of bacterial infection [13]; <39> the enzyme AiiA inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of pathogenic

bacteria [11]; <11> the isozymes AttM, AiiB, and AiiC inactivate the acyl-homoserine lactone quorom-sensing signaling and attenuate the virulence of *Erwinia carotovora* subsp. atroseptica strain 6276 on *Solanum tuberosum*, function and regulation of quorom-sensing signaling, overview [3]; <6, 12, 16, 17, 18, 19, 20, 43> the acylhomoserine lactones are inducers of quorom-sensing signaling [1]) (Reversibility: ?) [1, 2, 3, 6, 10, 11, 13, 16]

P N-acyl-(S)-homoserine

S N-butanoyl-L-homoserine lactone + H<sub>2</sub>O <7, 35, 38> (<7> high activity [10]; <35> the substrate regulates production of virulence determinants of the human pathogen *Pseudomonas aeruginosa* [13]) (Reversibility: ?) [10, 11, 13]

P N-butanoyl-L-homoserine (<35> product identification by mass spectrometry [13])

S N-decanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 5, 7, 38> (<4,7> high activity [9,10]) (Reversibility: ?) [5, 9, 10, 11]

P N-decanoyl-L-homoserine

S N-hexanoyl-(S)-homoserine lactone <15> (Reversibility: ?) [7]

P N-hexanoyl-(S)-homoserine

S N-hexanoyl-(S)-homoserine lactone + H<sub>2</sub>O <15> (<15> no activity with N-hexanoyl-(R)-homoserine lactone [7]) (Reversibility: ?) [7]

P N-hexanoyl-(S)-homoserine

S N-hexanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 7, 11, 38, 39, 40> (<7> best substrate [10]) (Reversibility: ?) [3, 9, 10, 11, 15]

P N-hexanoyl-L-homoserine (<38> product analysis of recombinant enzyme expressed in *Escherichia coli* strain JM109 [11])

S N-octanoyl-L-homoserine lactone + H<sub>2</sub>O <4, 7, 38> (<7> high activity [10]) (Reversibility: ?) [9, 10, 11]

P N-octanoyl-L-homoserine

S Additional information <1, 2, 3, 4, 5, 7, 9, 10, 13, 15, 35, 38, 39, 42> (<4> acyl-homoserine lactone quorom-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorom-quenching enzyme [9]; <1,2,3,9,10> acyl-homoserine lactone quorom-sensing signals play a key role in synchronizing virulence gene expression during bloodstream infections of mammals, the enzyme inactivates the AHL signaling by hydrolysis of the lactone ring thus acting as quorom-quenching enzyme [9]; <7,15> N-acyl homoserine lactone quorom-sensing signals are the vital elements of bacterial quorom-sensing systems, which regulate diverse biological functions, including virulence [7,10]; <5> N-acyl homoserine lactone quorom-sensing signals are the vital elements of bacterial quorom-sensing systems, which regulate diverse biological functions, including virulence, regulation quorom-sensing signaling and quorom-quenching in *Pseudomonas aeruginosa* [5]; <42> N-acyl homoserine lactone, AHL, quorom-sensing signals are the vital elements of bacterial quorom-sensing systems, which regulate diverse biological functions, including virulence and biofilm formation of Gram-negative bacteria, the enzyme deactivates

the signaling by degradation of AHLs via lactone ring hydrolysis [8]; <38,39> quorum-sensing is a signaling mechanism, that controls diverse biological functions, including virulence, via N-acylhomoserine lactone signal molecules in Gram-negative bacteria, overview [11]; <13> the enzyme is involved in inactivation of N-acylhomoserine lactone signaling in regulation of infection and virulence functions [12]; <4> substrate specificity, N-butanoyl-L-homoserine lactone is a poor substrate [9]; <7> substrate specificity, poor activity with non-acyl lactones and no activity with non-cyclic esters, but strong activity with all N-acyl-(S)-homoserine lactones of different chain length and nature, the amide group and the ketone at C<sub>1</sub> position of the substrates acyl chain might be important for substrate-enzyme interaction, overview [10]; <35> the substrate N-acyl-L-homoserine lactones show negligible enzyme-independent delactonization [13]] (Reversibility: ?) [5, 7, 8, 9, 10, 11, 12, 13]

P ?

#### Inhibitors

$\text{Ag}^+$  <7> (<7> complete inhibition at 0.2 mM [10]) [10]  
 $\text{Cr}^{3+}$  <7> (<7> 72% inhibition at 2 mM [10]) [10]  
 $\text{Cu}^{2+}$  <7> (<7> complete inhibition at 0.2 mM [10]) [10]  
EDTA <4> (<4> in vitro and in serum [9]) [9]  
 $\text{Fe}^{2+}$  <7> (<7> 48% inhibition at 2 mM [10]) [10]  
 $\text{Pb}^{2+}$  <7> (<7> 67% inhibition at 2 mM [10]) [10]  
TPEN <40> (<40> i.e. N,N,N,N-tetrakis-(2-pyridylmethyl)-ethylene-diamine [15]) [15]  
Additional information <7> (<7> enzyme is not affected by EDTA, 2,2-bipyridine, and *o*-phenanthroline at 2 mM [10]) [10]

#### Activating compounds

Additional information <7> (<7> enzyme is not affected by EDTA, 2,2-bipyridine, and *o*-phenanthroline at 2 mM [10]) [10]

#### Metals, ions

$\text{Ca}^{2+}$  <1, 2, 3, 4, 9, 10> (<1,2,3,4,9,10> activates, required [9]) [9]  
 $\text{Zn}^{2+}$  <6, 12, 15, 16, 17, 18, 19, 20, 35, 40, 43> (<15> dinuclear form, 2  $\text{Zn}^{2+}$  ions per enzyme molecule, preparation of the apoenzyme, which is inactive, and reconstitution with  $\text{Zn}^{2+}$  at pH 7.1 restoring about 60% of native enzyme activity, metal content determination, overview [7]; <35> metallohydrolase containing the conserved HXHXDH zinc-binding motif required for full enzyme activity [16]; <6, 12, 17, 18, 19, 20, 43> metallohydrolase containing the conserved zinc-binding motif [1]; <16> metallohydrolase containing the conserved zinc-binding motif, residues H106, H109, D108, and H169 of the strain 240B1 enzyme are required for  $\text{Zn}^{2+}$  binding and full enzyme activity, overview [1]; <40> the enzyme is a metallohydrolase and contains 2  $\text{Zn}^{2+}$  ions per enzyme molecule involved in catalysis in a dinuclear zinc-binding center involving residues H104, H106, H169 for coordination of the first  $\text{Zn}^{2+}$ , and H109, H235, and D108 for coordination of the second  $\text{Zn}^{2+}$ , determination of metal content of wild-type and mutant enzymes, AiiA has a very high affi-

nity for Zn<sup>2+</sup>, overview [15]; <15> the enzyme is a metalloprotein [7]) [1, 7, 15, 16]

Additional information <4, 7> (<4> no activation by Mg<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> [9]; <7> no effect by Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup>, the enzyme contains the putative zinc-binding motif 104HXHxDH109, but is no metallohydrolase since it does not contain and require Zn<sup>2+</sup> or other metal ions, only trace amounts of Zn<sup>2+</sup>, the 104HXHxDH109 sequence is a catalytic motif of the AHL-lactonase [10]) [9, 10]

#### Turnover number (min<sup>-1</sup>)

- 20.2 <7> (N-3-oxodecanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 22.2 <7> (N-3-oxooctanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 22.7 <7> (N-3-oxohexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 27.5 <7> (N-octanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 28.6 <7> (N-3-oxobutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 29.3 <7> (N-3-hydroxybutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 35.7 <7> (N-hexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 117 <15> (N-hexanoyl-(S)-homoserine lactone, <15> pH 7.4, 28°C, purified detagged recombinant enzyme [7]) [7]

#### Specific activity (U/mg)

- 0.012 <2> (<2> serum [9]) [9]
- 0.013 <3, 9> (<3,9> serum [9]) [9]
- 0.014 <10> (<10> serum [9]) [9]
- 0.016 <1> (<1> serum [9]) [9]
- 0.017 <4> (<4> serum, substrate N-3-oxododecanoyl-L-homoserine lactone [9]) [9]

Additional information <7, 15, 38> [7, 10, 11]

#### K<sub>m</sub>-Value (mM)

- 0.0014 <7> (N-3-oxodecanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 0.0023 <7> (N-3-oxooctanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 0.0026 <7> (N-octanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 0.003 <7> (N-3-oxohexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]
- 0.0038 <7> (N-hexanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]

0.0047 <7> (N-3-oxobutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]

0.0051 <7> (N-butanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]

0.0075 <7> (N-3-hydroxybutanoyl-L-homoserine lactone, <7> pH 7.4, 22°C, recombinant wild-type enzyme [10]) [10]

6.7 <15> (N-hexanoyl-(S)-homoserine lactone, <15> pH 7.4, 28°C, purified detagged recombinant enzyme [7]) [7]

Additional information <7, 11, 15> (<11> kinetics [3]; <7> kinetics and thermodynamics [10]; <15> steady-state kinetics, different enzyme constitutions [7]) [3, 7, 10]

#### pH-Optimum

6.5 <11, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> (<11, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> in vivo assay at [2,3]) [2, 3]

7.4 <1, 2, 3, 4, 9, 10, 15> (<1,2,3,4,9,10,15> assay at [7,9]) [7, 9]

8 <7> (<7> broad optimum of pH 7.0 to pH 9.0 [10]) [10]

#### pH-Range

6-9 <7> (<7> complete loss of activity at pH 5.5 and above pH 9.0, pH-profile [10]) [10]

#### Temperature optimum (°C)

22 <7> (<7> assay at [10]) [10]

25 <11> (<11> in vivo assay at [3]) [3]

28 <6, 12, 15, 16, 17, 18, 19, 20, 43> (<15> assay at [7]; <6, 12, 16, 17, 18, 19, 20, 43> in vivo assay at [1]) [1, 7]

37 <1, 2, 3, 4, 9, 10, 15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> (<1, 2, 3, 4, 9, 10> assay at [9]; <15, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37> in vivo assay at [2]) [2, 9]

#### Temperature range (°C)

6-37 <7> [10]

## 4 Enzyme Structure

#### Subunits

? <15, 38> (<15> x \* 29000, SDS-PAGE [7]; <15> x \* 29000, recombinant detagged enzyme, SDS-PAGE [7]; <38> x \* 30000, native enzyme, SDS-PAGE, x \* 36000, recombinant enzyme, SDS-PAGE [11]) [7, 11]

Additional information <7, 15, 38> (<38> AhLD contains the conserved HXDH-H-D motif required for full enzyme activity [11]; <7> analysis of secondary wild-type and mutant enzyme structures, comparison, overview [10]; <15> enzyme secondary structure analysis and oligomeric state, overview [14]) [10, 11, 14]

## 5 Isolation/Preparation/Mutation/Application

### Source/tissue

culture condition:N-3-oxododecanoyl-L-homoserine lactone/ammonium chloride-grown cell <38, 39> [11]

culture condition:N-decanoyl-L-homoserine lactone-grown cell <5> [5]

serum <1, 2, 3, 4, 9, 10> [9]

### Purification

<4> [9]

<7> (recombinant GST-tagged wild-type and mutant enzymes from Escherichia coli by glutathione affinity chromatography) [10]

<15> [7]

<15> (recombinant maltose binding protein-fusion enzyme from Escherichia coli strain DH5 $\alpha$  to over 95% purity, the fusion tag is cleaved off by TEV protease, the detagged enzyme is purified by DEAE ion exchange chromatography, and amylose affinity chromatography to remove traces of MBP, further purification by hydrophobic interaction chromatography in presence of 1.6-2.0 M ammonium sulfate, and dialysis) [7]

<35> (recombinant GST-tagged AiiA from Escherichia coli strain DH5 $\alpha$ ) [16]

<35> (recombinant GST-tagged AiiA from Escherichia coli strain DH5 $\alpha$  by glutathione affinity chromatography, the GST-atg is cleaved off by thrombin) [13]

<38> (partially from strain IBN110 by ammonium sulfate fractionation and two steps of ion exchange chromatography, full purification failed due to enzyme instability) [11]

<42> (recombinant His6-tagged maltose binding protein-fusion enzyme from Escherichia coli strain XL 1-Blue by amylose and nickel affinity chromatography and gel filtration, the fusion tag is cleaved off by TEV protease) [8]

### Crystallization

<15> (purified recombinant detagged enzyme, hanging drop vapour diffusion method, 10 mg/ml protein in 20% glycerol, 80 mM Tris-HCl, pH 8.5, 24% PEG 4000, and 160 mM MgCl<sub>2</sub>, in a ratio of 4:1 mixed with well solution, room temperature, 2 weeks, X-ray diffraction structure determination and analysis at 1.6 Å resolution using single-wavelength anomalous dispersion phasing, modeling) [14]

<40> (native and selenomethionine-labeled enzyme, free or in complex with L-homoserine lactone, X-ray diffraction structure determination and analysis at 1.7-2.0 Å resolution, structure modeling) [15]

<42> (purified detagged recombinant native and selenomethionine-labeled enzyme, sitting drop vapour diffusion method, 7 mg/ml protein in 20 mM Tris-HCl, pH 7.5, mixed with and equilibrated against the reservoir solution containing 0.1 M Tris-HCl, pH 8.0, 30% PEG 4000, and 0.2 M MgCl<sub>2</sub>, 21°C, 5 days, X-ray diffraction structure determination and analysis at 2.0 Å resolution) [8]

**Cloning**

- <6> (functional expression of AiiA lactonase in *Burkholderia thailandensis*) [4]
- <6> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <7> (gene aiiA, expression in *Burkholderia cepacia*, an organism of the *Burkholderia* complex, from a broad-host range plasmid, the expression abolishes or greatly reduces the accumulation of N-acyl-homoserine lactone molecules in the *Burkholderia cepacia* complex strains, phenotype, overview) [6]
- <7> (gene aiiA, expression of wild-type and mutant enzymes in *Escherichia coli* as GST-tagged proteins) [10]
- <11> (genes attM, aiiB, and aiiC, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli*) [3]
- <12> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <14> (functional expression of AiiA lactonase in *Burkholderia thailandensis*) [4]
- <15> (expression in *Escherichia coli* strain DH5 $\alpha$ ) [7]
- <15> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]
- <15> (gene aiiA, expression in *Escherichia coli* strain DH5 $\alpha$  fused to the maltose binding protein with the TEV protease cleavage site ENLYFQ\*G) [7]
- <16> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <17> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <18> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <19> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <20> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]
- <21> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]
- <22> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]
- <23> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]
- <24> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<25> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<26> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<27> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<28> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<29> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<30> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<31> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<32> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<33> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<34> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<35> (expression of GST-tagged AiiA containing a thrombin cleavage site in *Escherichia coli* strain DH5 $\alpha$ , construction of transgenic *Solanum tuberosum* and *Nicotiana tabacum* plants expressing gene aiiA) [13]

<35> (gene aiiA, DNA and amino acid sequence determination and analysis, expression of GST-tagged AiiA in *Escherichia coli* strain DH5 $\alpha$ , expression in *Erwinia carotovora* strain SCG1 leading to reduction of autoinducer release thereby decreasing extracellular pectolytic enzyme activities, and attenuating pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco) [16]

<36> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<37> (gene aiiA, DNA and amino acid sequence determination and analysis, phylogenetic dendrogram, subcloning and overexpression in *Escherichia coli* strain DH5 $\alpha$  and BL21(DE3)) [2]

<38> (gene ahLD, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strains DH5 $\alpha$ , BL21(DE3) as His-tagged enzyme, and JM109) [11]

<39> (gene ahIK, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain JM109) [11]

<40> (expression of wild-type and mutant enzymes as His-tagged maltose binding protein-fusion proteins) [15]

<42> (gene aiiA, expression of His6-tagged maltose binding protein-fusion enzyme with the TEV protease cleavage site in *Escherichia coli* strain XL 1-Blue) [8]

<43> (gene aiiA, DNA and amino acid sequence determination and analysis, expression in *Escherichia coli* strain DH5 $\alpha$ ) [1]

### Engineering

D108A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn<sup>2+</sup> content compared to the wild-type enzyme [15]) [15]

D108E <35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant shows 90.8% of wild-type enzyme activity [16]) [16]

D108S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [16]; <7> site-directed mutagenesis, the mutant shows no activity, and altered secondary structure [10]) [10, 16]

D108S/H109S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]

D191A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn<sup>2+</sup> content compared to the wild-type enzyme [15]) [15]

D191S <16> (<16> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [1]) [1]

D236S <16> (<16> site-directed mutagenesis, the mutation does not affect the enzyme activity [1]) [1]

H104L/H106L/D108L <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 61.4% of wild-type enzyme activity [16]) [16]

H104L/H106L/D108L/H109L <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 37.9% of wild-type enzyme activity [16]) [16]

H104S <35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant is as active as the wild-type enzyme [16]) [16]

H104S/H106S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, the mutant shows 51.1% of wild-type enzyme activity [16]) [16]

H104S/H106S/D108S/H109Sc <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]

H104S/H106S/H109S <35> (<35> site-directed mutagenesis, mutation of zinc-binding motif residues, inactive mutant [16]) [16]

H106S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, the mutant shows 61.4% of wild-type enzyme activity [16];

<7> site-directed mutagenesis, the mutant shows 53.5% of wild-type enzyme activity, and altered secondary structure [10]) [10, 16]

H109A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn<sup>2+</sup> content compared to the wild-type enzyme [15]) [15]

H109S <7, 35> (<35> site-directed mutagenesis, mutation of a zinc-binding motif residue, inactive mutant [16]; <7> site-directed mutagenesis, the mutant shows no activity, and altered secondary structure [10]) [10, 16]

H169S <7> (<7> site-directed mutagenesis, the mutant shows 53.1% of wild-type enzyme activity, and altered secondary structure [10]) [10]

H235A <40> (<40> site-directed mutagenesis, the mutant enzyme shows a reduced Zn<sup>2+</sup> content compared to the wild-type enzyme [15]) [15]

H235S <16> (<16> site-directed mutagenesis, the mutation does not affect the enzyme activity [1]) [1]

Additional information <5, 6, 14, 35> (<35> construction of transgenic Solanum tuberosum and Nicotiana tabacum plants via Agrobacterium tumefaciens transfection with Bacillus sp. gene aiiA inserted into their genome, the transgenic plants express the recombinant aiiA gene and show increased AHL-lactonase activity compared to the wild-type plants, as well as enhanced resistance to plant pathogen *Erwinia carotovora*, the recombinant enzyme might be trapped in microsomes [13]; <14> expression of AiiA lactonase in Burkholderia thailandensis completely abolishes the accumulation of N-decanoyl-L-homoserine lactone and N-octanoyl-L-homoserine lactone, reduces N-hexanoyl-L-homoserine lactone levels, alters both swarming and twitching motility, causes a significant increase in generation time, and affects carbon metabolism, but the AHL lactonase activity does not enhance β-hemolytic activity in the transgenic bacteria, overview [4]; <6> expression of AiiA lactonase in Burkholderia thailandensis reduces the concentrations of N-decanoyl-L-homoserine lactone, N-octanoyl-L-homoserine lactone, and N-hexanoyl-L-homoserine lactone, alters both swarming and twitching motility, and caused fluctuations in carbon utilization, but the AHL lactonase activity does not enhance β-hemolytic activity in the transgenic bacteria, overview [4]; <5> transposon mutants of quiP are defective in growth when *Pseudomonas aeruginosa* is cultured on decanoyl-(S)-homoserine lactone as sole carbon and energy source, the growth-defect mutant strain can be rescued by complementation with a functional copy of quiP, constitutive expression of quiP leads to decreased accumulation of the quorum signal molecule N-3-oxododecanoyl-L-homoserine lactone [5]) [4, 5, 13]

## 6 Stability

### Temperature stability

37 <7> (<7> purified recombinant wild-type enzyme, completely stable below [10]) [10]

45 <7> (<7> purified recombinant wild-type enzyme, inactivation, 2 h [10]) [10]

95 <4> (<4> inactivation within 3 min [9]) [9]

**General stability information**

<38>, the enzyme is instable during purification [11]

**Storage stability**

<7>, 4°C or 21°C, purified recombinant enzyme, 10 days, 99% remaining activity [10]

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